

DESCRIPTION OF A ZOOCHLORELLA-BEARING FORM OF
EUPLOTES, *E. DAIDALEOS* N. SP. (CILIOPHORA,
HYPOTRICHIDA)

WILLIAM F. DILLER AND DEMETRIUS KOUNARIS

Department of Biology, University of Pennsylvania, Philadelphia, Pa. 19104

Marine and fresh-water species of *Euplotes* are numerous and widely distributed throughout the world. Reviews and historical accounts have been given by Kahl (1932), Pierson (1943), Bovee (1957), Tuffrau (1960), Wichterman (1964) and others. In spite of a great deal of morphological, genetic and cultural work, the taxonomy of this genus is still in an unsettled condition due to variability within species, conjugation between recognized different species, failure of morphologically similar forms to mate and utilization of perhaps unreliable criteria in species differentiation. Moreover, it seems reasonable to believe, as Bovee has suggested, that morphological variation and speciation have followed after reproductive and physiological variation in this genus; hence, the obvious difficulty of dealing with this interesting group of ciliates and the need for the employment of a variety of characters in the recognition of species. A great advance in the analysis of the genus *Euplotes* was made by Tuffrau in his application of the following combination of characters: (1) the number of latero-dorsal kineties, (2) the pattern of the argyrome on the dorsal surface, (3) the number of frontoventral cirri and (4) the shape of the meganucleus in the vegetative state. He based his own extensive revision of the genus on these specific traits. Other students would extend diagnostic criteria to further features, particularly characters relating to the peristome (buccal cavity). Undue reliance on argyrome traits alone can be misleading. For example, the number of kineties in a given species may be variable, as in *E. tuffraui* (Berger, 1965), *E. vannus* (Dusenberry, 1966) and one stock of *E. crassus* (Dusenberry, 1966). In addition, there may be several species possessing the same number of kineties and a somewhat similar dorsal argyrome pattern (Tuffrau, 1960). Recently evolved species may have argyrome patterns very similar to, if not identical with, the ancestral forms, so that it may be very difficult to decide whether or not a given variant is a separate species. The most recently described species, to the writers' knowledge, are *E. leticiensis* Bovee (1957), *E. neapolitanus* Wichterman (1964) and *E. tuffraui* Berger (1965). Borrer (1962) has re-described *Euplotes minuta* Yocum. Wichterman reports (1964, pp. 368-369) that "Vacelot (1961) described what he believed to be a new marine species which he named *E. psammophilus*, from *Amphioxus*-sand near Marseilles; but his brief description and poor figure are inadequate to set the species on a firm foundation."

MATERIALS AND METHODS

Collections of samples from a small, permanent, artificial fresh-water pond in the Biological Gardens on the campus of the University of Pennsylvania have con-

sistently yielded over the past seven years specimens of a green species of *Euplotes* which is here named and described as *E. daidaleos* n. sp.¹ Not every collection was positive but there has been no difficulty in securing material from this source. The green *Euplotes* is never present in large numbers in a freshly collected sample and it is often found together with one or more colorless species of *Euplotes* from which it is easily distinguishable because of its different size and its characteristic bright green color. However, when there are few algae in *E. daidaleos* it can be confused with the colorless species which may have ingested green organic food. The green species is not difficult to maintain in the laboratory and thrives on the usual ciliate culture media—hay infusion, Cerophyl infusion, malted milk, powdered milk, rice grains in boiled pond water, etc.

Animals were studied alive, slowed down by 0.05% nickel sulfate or by methylcellulose, as well as in fixed and stained preparations. Many cytological techniques were employed, the most useful being (1) formalin-vapor fixation followed by Bouin's and stained by the Holmes silver technique (Figs. 1–3), (2) the Chatton-Lwoff silver impregnation (Figs. 4, 5 and 8), and (3) Perenyi fixation with carmine-piconigrosin staining (Figs. 6 and 7). All three methods have been described in detail in a previous paper (Diller, 1966).

RESULTS

Size and shape

This fresh-water species averages 92 μ in body length and 57 μ in width, for vegetative non-dividing individuals. A range of 77 μ to 119 μ in length and 43 μ to 80 μ in breadth is encountered. Dividing animals are somewhat larger, as might be expected, averaging 102 μ in length and 59 μ in width. Exconjugants are shorter and more rounded, averaging 80 μ long and 55 μ wide. *E. daidaleos* is definitely smaller than the common colorless species with which it is commonly found in nature. As is true of all species of *Euplotes*, *E. daidaleos* has a very strongly flattened body and is oval to rounded in face view. The anterior margin, bearing a low collar, is truncated, while the posterior end tends to be bluntly pointed (Figs. 1, 2 and 3). The right anterior margin is straighter than the corresponding corner on the left side which is more rounded. The dorsal surface is slightly convex and bears a constant number of ridges. The ventral surface is flatter and also is equipped with ridges of a characteristic nature. The collar carries the transverse anterior adoral membranelles.

Surface organelles

The peristome is capacious and tapers to a narrow funnel slightly behind the middle of the body, at the cytostome (Figs. 1, 2 and 8). It is bordered on its left and anterior margins by the adoral zone of membranelles (AZM). These are small and close-set in the cytostomal region, becoming wider and larger anteriorly.

¹ The much-studied genus *Euplotes* with its numerous species and many synonyms offered a problem in the selection of an appropriate name for a new species. The term "daidaleos" was finally selected after reference to Brown (1954). He (p. 742) gives the meaning of the Greek word daidaleos as "dappled, spotted." It has an additional meaning (daidalos, p. 104): "cunningly or skilfully made in the manner of Daedalus, the Athenian artificer." The species name seemed fitting, especially so because of the Greek origin of the word *Euplotes*.

There are approximately 40–45 units constituting this structure. Posteriorly, the AZM is opposed by the prominent band of cilia in the mouth area (Figs. 1 and 2) which is known as the undulating or paroral membranelles. The exact conformation of the peristomal cavity is a little difficult to determine but can be analyzed satisfactorily from silver and from picronigrosin preparations. The right edge is

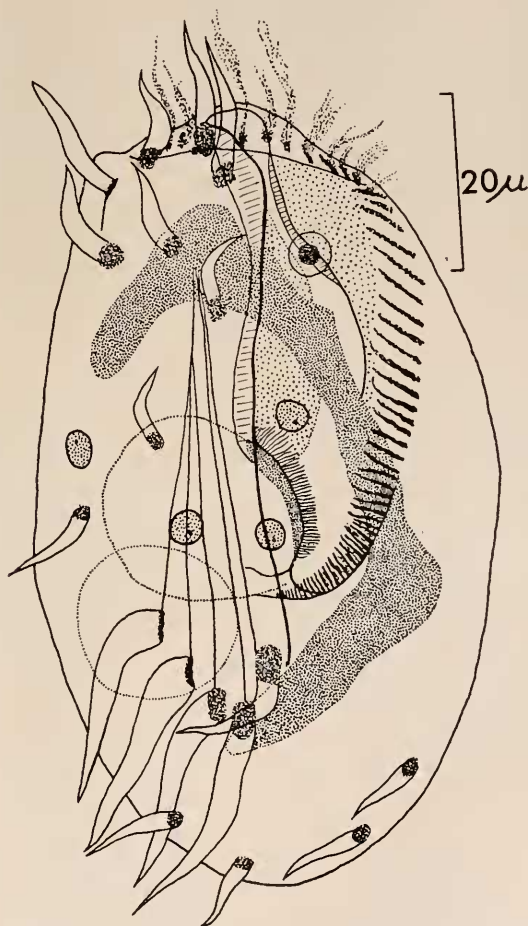
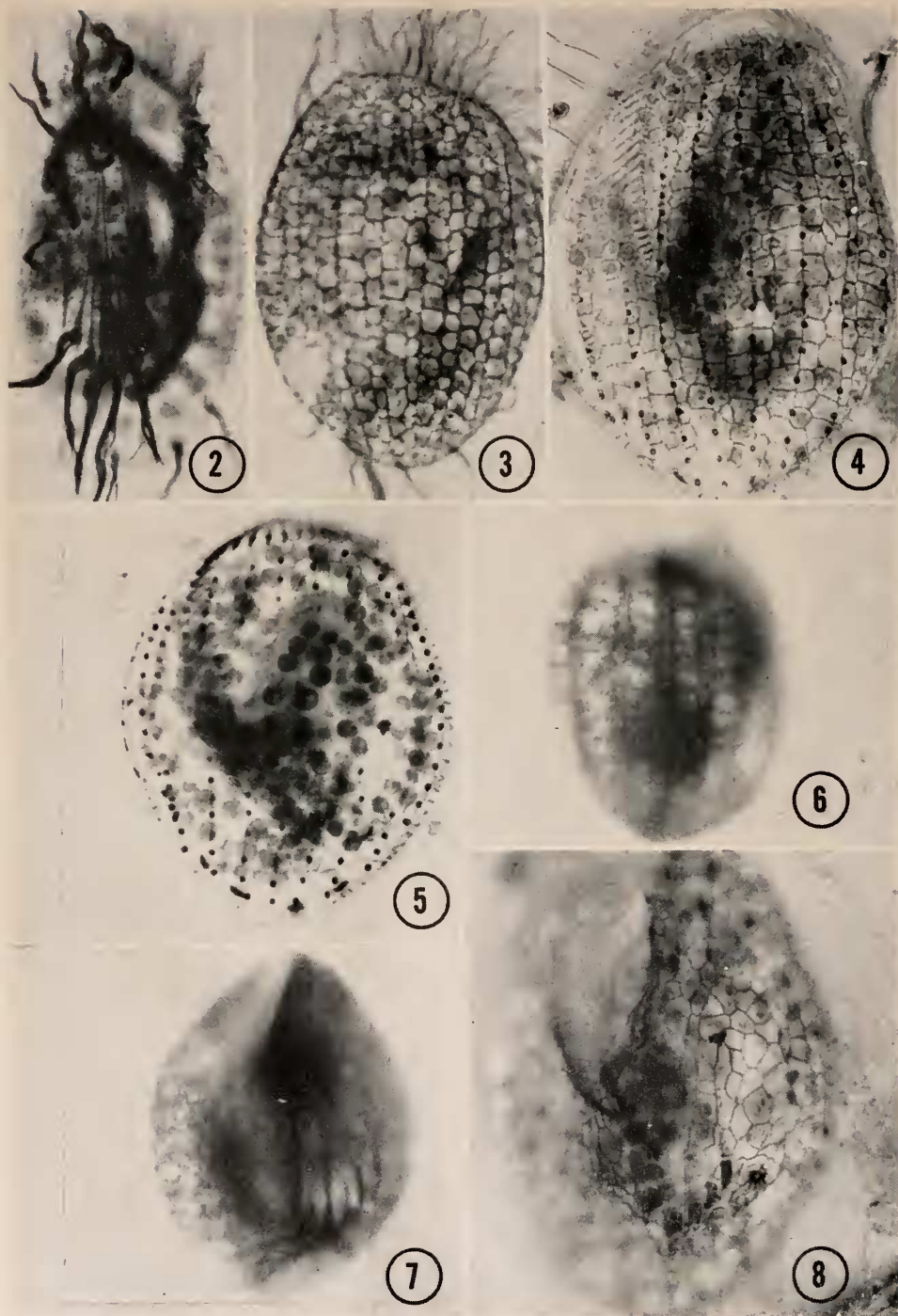


FIGURE 1. Semi-diagrammatic drawing of *Euplotes daidaleos* n. sp. from the ventral side, showing structural features. Taken from a fixed specimen, stained with Holmes silver. Note the collar at the anterior margin of the body bearing some of the adoral membranelles (AZM) which continue along the left wall of the buccal cavity (peristome) to terminate at the cytostome; the paroral membranelles near the base of the peristome on its right wall; the peristomal plate extending along the left wall of the peristome; a bulge on the opposite wall of the peristome occupying a considerable portion of the roof of it; an irregular channel between these two thickened areas of the peristomal roof; the endoplasmic sac (corresponding to the level of the paroral membranelles); the contractile vacuole just posterior to the sac; the meganucleus; the micronucleus; the eighteen cirri (six frontals, three smaller ventrals, five anals and four caudals); the "neuromotor" fibers from the bases of the anal cirri and the four zoochlorellae in the cytoplasm.



FIGURES 2-8.

almost straight, being formed by a continuation of the heavy ridge which runs anteriorad from the left side of the medial anal cirrus. This border is undercut by a shallow furrow so as to form a narrow longitudinal lip which is usually a little wider at its anterior and posterior limits than in its center. The lip continues posteriorly over the paroral membranelles to form the posterior border of the peristome, the funnel of the mouth. Along a good part of the right wall of the peristome is a shallow bulge (Fig. 1, stippled) which continues over part of the roof of the peristomal cavity from near the anterior end to about the middle of the paroral membranelles. Opposite this bulge is another thickening of the left wall and roof of the peristome, the so-called peristomal plate (Fig. 1, also stippled). It bears a very small lip. A trough or channel is formed in the roof of the peristome by the space between these two thickenings. The channel is expanded anteriorly at the level of the collar and also posteriorly in the region where actual ingestion occurs. In other species of *Euplotes* we have seen small food organisms entrapped in the anterior expansion which seems to serve as a food-collecting mechanism. However, we have not noticed any collection of food material in this structure in *E. daidaleos*.

The 18 cirri are very constant in number and position. In comparison with colorless species, one has the impression that those of *E. daidaleos* are longer and slenderer. Very rarely five caudal cirri (instead of four) are present. These may represent reorganizations from division of the cell. Also, in exconjugants (at certain stages) only five instead of six frontal cirri are present. The usual "biological variation" can account for the infrequent cirral anomalies which affect the caudals, mainly. The number and position of the cirri are as follows: six frontals in three rows of two each, three ventrals in an oblique row on the right ventral

EXPLANATION OF FIGURES 2-8

Euplotes daidaleos. Photomicrographs. Specimens shown in Figures 2 and 3 were fixed in formalin vapor and Bouin's, followed by the Holmes silver technique. Figures 4, 5 and 8 are specimens fixed in Champy, DaFano and treated with the Chatton-Lwoff silver impregnation. Figures 6 and 7 are exconjugants fixed in Perenyi's fluid and stained by Grenacher's alcoholic borax carmine and picronigrosin.

FIGURE 2. Ventral view. Typical individual showing the locations of all of the eighteen cirri, the shape of the meganucleus, the fibrils from the anal cirri, the AZM, the paroral membranelles and some of the zoochlorellae (approx. 50). $\times 700$.

FIGURE 3. Ventral view, with the dorsal argyrome in focus. The alternating rows of narrower and wider polygons or "compartments" are easily seen. The dorsal bristles are not prominent. $\times 615$.

FIGURE 4. Dorsal view. Most of the rows of dorsal bristles are visible—located in meridians on the right side of the wider "compartment" rows. $\times 650$.

FIGURE 5. Dorsal view. All nine rows of latero-dorsal bristles are visible. The zoochlorellae are prominent, as are the elongated basal plates of the four caudal cirri. $\times 645$.

FIGURE 6. Dorsal view. The rows of narrow polygons contain or overlie protoplasmic constituents (probably mitochondria) which are thus organized differently from their condition in the wider "compartment" rows so as to give a banded organization to the dorsal cortex. $\times 600$.

FIGURE 7. Dorsal view, with the ventral surface in focus. The stained granules noted in Figure 6 are differentially distributed in the ventral cortex also, outlining the ventral ridges. $\times 625$.

FIGURE 8. Dorsal view, with focus on the ventral surface to show the nature of the ventral argyrome pattern. To the right of the bases of the anal cirri is the contractile vacuole pore. $\times 690$.

surface, five large anals (four in line on the right side and the medial one slightly anterior to the last of this line) and four small caudals. The two right caudals regularly tend to be fimbriated.

Ridges are present on both dorsal and ventral surfaces. On the latter there are six ridges supporting the grooves in which the anal cirri lie. The ridges are of varying lengths and prominence (Fig. 7). Starting from the right side, nos. 1, 4 and 6 are the longest. The latter participates in the formation of the major ridge which becomes the right wall of the buccal cavity. The ridges on the dorsal side are definite, parallel, equidistant and each bears a row of dorsal bristles. More will be said about these "latero-dorsal" bristle rows in connection with the discussion of the argyrome.

The argyrome

Various silver techniques (Figs. 2, 3, 4, 5 and 8) and, under certain conditions, the picronigrosin stain (Fig. 6) demonstrate the "latero-dorsal kineties," the dorsal bristles and associated structures which may be interpreted as either polygons, fibrils or a meshwork. Exactly what is brought out is somewhat uncertain: kinetosomes, pellicular structures or actual cortical protoplasmic elements. Probably a variety of structures is indeed impregnated and caution is required in interpretation. However, with the use of the standard Chatton-Lwoff technique, *e.g.*, in Figures 4, 5 and 8, striking, reproducible and exceedingly useful preparations can be produced so as to compare and separate different forms of *Euplotes*. Tuffrau has made extensive use of this silver technique in his admirable study of the genus. *E. daidaleos* possesses nine latero-dorsal rows of bristles (Figs. 4 and 5 particularly). Eight of these kineties are on the dorsal surface, the ninth definitely on the ventral surface. In specimens well flattened in gelatine all nine kineties may be visible at practically the same focus. The number of kineties is remarkably constant in *E. daidaleos*: in hundreds of specimens carefully studied only one showed a discrepancy from this number, nine. The number of kinetosomes per row is also fairly constant. Starting with the ventral row, then going to the dorsal side and proceeding to the right edge we have counted the following numbers of bristles per row: 13-16, 7-17, 15-18, 15-19, 17-20, 16-18, 16-18, 13-19, 10-19. The total number of bristles per animal varied from 123 to 158 in the specimens counted. Between the kineties—kinetosomes seem to lie on a "fibril"—are stainable lines (Figs. 4 and 5) which look like cross-connecting fibrils. With the picronigrosin technique (Fig. 3) one gets the impression that this configuration may be shallow polygons of alternating wider and narrower rows (Figs. 3 and 6) of pellicular or of superficial cortical material. The dorsal bristles always lie to the right of a wide row and to the left of a narrow row of these polygons. It would appear that the wider and narrower rows may represent some real cytoplasmic difference, since, with the picronigrosin stain (Fig. 6), there is a differential staining reaction, the narrow rows taking up the blue stain much more intensely than the wide rows do. Probably, the mitochondria are being stained and this banded effect represents a differential distribution of the mitochondria. One can think of no logical explanation for this condition. On the ventral surface the mitochondria in the ridges take up the picronigrosin intensely (Fig. 7). The argyrome pattern of the ventral

surface (Fig. 8) is more irregular and finer (including the roof of the peristome) than it is on the dorsal surface. As is characteristic of all species of *Euplotes*, the meshwork immediately surrounding the contractile vacuole pore, to the right of the anal cirri (Fig. 8), consists of much smaller polygons.

Internal structures

The shape of the C-shaped meganucleus is somewhat variable and not particularly distinctive for *E. daidaleos*. Typically, the anterior arm bends backward but the posterior arm does not curve forward to meet it, so that a symmetrical C is not formed (Figs. 1 and 2). Usually the anterior arm extends forward into a small hump as it becomes drawn out in the main body of the organelle. The position of the micronucleus is very constant, at the anterior curvature of the meganucleus slightly dorsal to it and between it and the AZM. It is a small vesicular structure less than $5\ \mu$ in diameter enclosing a homogeneous endosome.

The presence of symbiotic algae in the cytoplasm of *E. daidaleos* is very characteristic and perhaps a constant feature of this species. The zoochlorellae vary in number from a very few (Fig. 1 contains four) to perhaps 100 (Figs. 4, 5 and 8). Their size ranges from 3 to $5\ \mu$ in diameter. When a culture is dividing rapidly the number of algae is less per host than when the population is static. We do not know whether this species can normally exist without their customary algae; if so, whether they can reacquire their symbionts from the medium. In clonal cultures, all of the individuals possess at least some zoochlorellae under normal conditions. When colorless *Euplotes* and *E. daidaleos* are mixed together, both colorless and green individuals can be recognized as such for many weeks. Just as *Paramecium bursaria* can be freed of its algae, so it might be expected that *E. daidaleos* can be sterilized of its zoochlorellae. However, critical studies have not yet been made on these matters of obligatory symbiosis, mode of "infection" and survival in the absence of algae. It should be noted that exconjugants frequently contain more algae than their non-conjugated associates. This can be explained on the basis of a long reorganization interval between conjugation and the first postconjugant fission during which the algae divide and are not diluted by division of their host cell.

Many of our Holmes silver preparations bring out the five long internal fibers originating at the bases of the anal cirri and converging at the anterior end in the region of the medial frontal cirri (Figs. 1 and 2), as well as other fibers associated with other cirri. We have not been able to detect any structure which could be interpreted as a neuromotorium.

The food material appears to be bacteria, algae, flagellates and small ciliates of the pleuronematid type. Some cultures show bacteria-like bodies in the cytoplasm which resemble Kappa particles in *Paramecium*. They are quite distinct from the mitochondria and are often sharply demonstrated by the Holmes technique. A prominent endoplasmic sac (Fig. 1) is present on the right side of the body. Its left side appears to be very intimately associated with the bases of the paroral membranelles.

Just posterior to the endoplasmic sac is the contractile vacuole (Fig. 1). Its external pore is a fixed spot on the ventral surface.

Life cycle

Conjugation has been observed frequently in non-isolated mass cultures of *E. daidaleos* and in cultures started from a few (8–10) individuals from wild cultures. Studies of the correlation of ciliary and nuclear development in the life cycle of this species have been published elsewhere (Kounaris, 1964; Diller, 1966). These report binary fission and conjugation. Encystment in this species has never been seen by us.

DISCUSSION

Colored species of *Euplotes* have been described as far back as Ehrenberg (1840) who named a form from Berlin *E. viridis*. Stein (1859) presented three figures of forms he called *Euplotes patella*, noting that very frequently their cytoplasm is more or less thickly filled with bright green chlorophyll bodies. He referred to one of these as "disc-forms." Apparently he felt that there were several types of chlorophyll-bearing *Euplotes*. Stein identified Ehrenberg's *Euplotes viridis* from Berlin as most probably nothing more than the chlorophyll-bearing form of *E. patella*. Pierson, in her review of species of *Euplotes* closely related to *Euplotes patella*, makes no reference to green *Euplotes*. However, Kahl (1932) listed three colored types: all "formae" of *E. patella*. These are forma *typicus*, 80–100 μ , mostly with zoochlorellae; forma *latus* 90–120 μ , often with zoochlorellae; forma *alatus*, broad form with zoochlorellae. Obviously, he recognized different forms as capable of harboring zoochlorellae. Tuffrau, in his revision of the genus *Euplotes*, does not mention the occurrence of zoochlorella-bearing forms and dismisses Kahl's alga-bearing formae, together with Ehrenberg's *E. viridis*, as synonyms of *E. patella*. This decision was made largely on the basis of culture work by Pierson and himself which indicated intra-clonal variation of *E. patella*.

Tuffrau lays particular emphasis on the distinctiveness of the dorsal argyrome pattern of *E. patella*: the alternating wide and narrow polygonal rows. *E. daidaleos* has a dorsal argyrome pattern similar to *E. patella*—perhaps not quite as regular as Tuffrau has shown. His size of *E. patella*—105–145 μ —is greater than we have found for *E. daidaleos*. However, Pierson's size of *E. patella*—90 by 52 μ —is practically the same as for *E. daidaleos*. Kahl's *E. patella* is larger.

Most of the published figures and descriptions of *Euplotes* allow for some uncertainty with regard to the detailed structure of the buccal cavity: its wall, relative length and curvature, lips, bulges, channels, plates and membranelles. It is uncertain how similar, or dissimilar, *E. daidaleos* is to other species with respect to the features of the buccal cavity but it does not seem to fit either Pierson's or Tuffrau's description of *E. patella* exactly. Pierson has emphasized the desirability of including these features in the diagnostic determination of species of this genus.

Clearly, *E. daidaleos* is closely related to *E. patella* but on the basis of difference in body shape, constant possession of zoochlorellae, smaller size and details of the structure of the peristome, *E. daidaleos* is considered to be a distinct species, hitherto undescribed.

SUMMARY

1. *Euplotes daidaleos* n. sp. is described as a hitherto unrecognized species. It is a fresh-water form found in Philadelphia, Pa., and appears to be closely related

to *E. patella*, differing from it in shape and size, in the possession of zoochlorellae and possibly in the structure of the peristome.

2. Diagnostic characteristics: Fresh-water. Contains zoochlorellae (few to 100). Average length 92 μ , width 57 μ . Body flattened, oval in face view; right anterior margin straighter than the more convex shoulder on the left side; posterior end bluntly pointed. Buccal cavity (peristome) extending slightly beyond middle of body. Adoral membranelles approximately 40, arranged in a smooth curve terminating on the right edge of the collar. Right edge of the peristome an almost straight wall originating as an extension of the ventral ridge at the left of the anal cirri; undercut so as to form a narrow lip, anterior to the paroral membranelles; attached to it and the dorsal wall of the buccal cavity is a low bulge partly occluding the cavity. Between this bulge and the elongated triangular peristomal plate on the left anterior side of the buccal cavity is a trough or channel expanded anteriorly as well as posteriorly in the region of the paroral membranelles. The latter delimit the endoplasmic sac on the right. Eighteen cirri: six frontals, three ventrals, five anals and four caudals, two of the latter on the right side tending to be fimbriated. Meganucleus C-shaped. Nine latero-dorsal rows of bristles, corresponding in position to the dorsal ridges (the left one distinctly ventral). Between each dorsal "kinety" are two rows of alternating wider and narrower polygons, revealed by silver impregnation techniques. The dorsal bristles lie on the right border of the wide rows and to the left of the narrow rows.

3. Conjugation is common in the stocks of *E. daidaleos* examined. Encystment has not been found.

LITERATURE CITED

- BERGER, J., 1965. The infraciliary morphology of *Euplotes tuffraui*, n. sp. *Protistologica*, 1: 17-32.
- BORROR, A. C., 1962. *Euplotes minuta* Yocum (Ciliophora, Hypotrichida). *J. Protozool.*, 9: 271-273.
- BOVEE, E. C., 1957. *Euplotes leticiensis*, n. sp., from the Letician Drainage into the Amazon River. *J. Protozool.*, 4: 124-128.
- BROWN, ROLAND W., 1954. Composition of Scientific Words. Pub. by the author; George W. King Printing Co., Baltimore, Md.
- DILLER, W. F., 1966. Correlation of ciliary and nuclear development in the life cycle of *Euplotes*. *J. Protozool.*, 13: 43-54.
- DUSENBERRY, P. A., 1966. Genetics of *Euplotes*. M.S. Thesis in Biology, Library, Univ. of Pennsylvania, Philadelphia, Pa.
- EHRENBERG, C. G., 1840. Monatsber. der Berl. Acad. von 1840. S. 200.
- KAHL, A., 1932. Urtiere oder Protozoa. I. Wimpertiere oder *Ciliata* (*Infusoria*). In: F. Dahl's Die Tierwelt Deutschlands, Gustav Fischer, Jena, Teil 25, 399-650.
- KOUNARIS, D., 1964. Conjugation in a green *Euplotes* with special reference to the kinetosomal activity. M.S. Thesis in Biology, Library, Univ. of Pennsylvania, Philadelphia, Pa.
- PIERSON, B. E., 1943. A comparative morphological study of several species of *Euplotes* closely related to *Euplotes patella*. *J. Morph.*, 72: 125-165.
- STEIN, F., 1859. Der Organismus der Infusionsthier. I. Allgemeine Theil und Naturgeschichte der Hypotrichen Infusionsthier. Leipzig.
- TUFFRAU, M., 1960. Révision du genre *Euplotes*, fondée sur la comparaison des structures superficielles. *Hydrobiologia*, 15: 1-77.
- WICHTERMAN, RALPH, 1964. Description and life cycle of *Euplotes neapolitanus* sp. nov. (Protozoa, Ciliophora, Hypotrichida) from the Gulf of Naples. *Trans. Amer. Micros. Soc.*, 83: 362-370.